

Appl. No. 10/693,428  
Amendment dated February 26, 2007  
Reply to Advisory Action of January 22, 2007

RECEIVED  
CENTRAL FAX CENTER

FEB 26 2007

**Listing of Claims:**

The following listing of the claims replaces all previous versions.

1. (Currently Amended) A method of preparing an RNA sample substantially free of genomic DNA, comprising the following steps:
  - (a) forming a lysate from a biological sample;
  - (b) removing essentially all genomic DNA from the lysate of (a) therein forming a sample preparation;
  - (c) forming an RNA-containing precipitate by adding an organic solvent to the sample preparation of (b);
  - (d) contacting ~~an RNA isolation membrane column with~~ said RNA-containing precipitate of (c) with a polymeric membrane having a particle retention of up to about 10  $\mu$ m, wherein said ~~column comprises a polymeric membrane acting~~ acts as a passive physical barrier to said RNA-containing precipitate and ~~retaining~~ retains the RNA-containing precipitate for purification; and
  - (e) collecting said RNA-containing precipitate from said membrane, wherein said RNA-containing precipitate is substantially free of said genomic DNA.
2. (Canceled)
3. (Previously presented) The method of claim 1, wherein said polymeric membrane is selected from the group consisting of polysulfone treated with hydroxypropylcellulose, poly(vinylidene fluoride), nylon, nitrocellulose, polysulfone, polysulfone and poly(vinylpyrrolidone), poly(vinylpyrrolidone), and composites thereof.
4. (Previously presented) The method of claim 1, wherein said membrane has a particle retention ranging from about 0.1  $\mu$ m to about 10  $\mu$ m.

Appl. No. 10/693,428  
Amendment dated February 26, 2007  
Reply to Advisory Action of January 22, 2007

5. (Previously presented) The method of claim 1, wherein said step (b) is accomplished by using a pre-filtration technique.
6. (Original) The method of claim 1, wherein said lysate is formed employing a lysis buffer comprising a chaotropic agent.
7. (Original) The method of claim 6, wherein said chaotropic agent is selected from a group consisting of guanidine isothiocyanate, ammonium isothiocyanate, guanidine hydrochloride, and combinations thereof.
8. (Original) The method of claim 7, wherein said chaotropic agent is at a concentration ranging from about 0.5 M to about 5.0 M.
9. (Previously presented) The method of claim 1, wherein said biological sample is selected from the group consisting of animal tissues, plant tissues, animal cells, and plant cells.
10. (Previously presented) The method of claim 9, wherein said biological sample is selected from a group consisting of blood, urine, hair, skin, muscle, bone, bodily fluids, and organ extracts.
11. (Previously presented) The method of claim 1, wherein step (e) is followed by treating the precipitate with DNase.
12. (Original) The method of claim 1, wherein said precipitate comprises RNA essentially free of DNA.

Appl. No. 10/693,428  
Amendment dated February 26, 2007  
Reply to Advisory Action of January 22, 2007

13. (Original) The method of claim 1, wherein said lysate is formed using a lysis buffer comprising  $\beta$ -mercaptoethanol.
14. (Original) The method of claim 1, wherein said organic solvent is an alcohol selected from the group consisting of methanol, ethanol, isopropanol and combinations thereof.
15. (Original) The method of claim 1, wherein said precipitate is washed following step (d) with a wash solution comprising an organic solvent.
16. (Previously presented) The method of claim 15, wherein said wash solution comprises ethanol and a buffering agent to maintain a pH from about 6 to about 9.
17. (Previously presented) The method of claim 16, wherein said wash solution comprises: (a) from about 0.2 to about 2 M guanidine; (b) from about 5 to about 25% ethanol; and (c) a buffering agent to maintain a pH from about 6 to about 9.
18. (Previously presented) The method of claim 16, wherein said wash solution comprises: (a) from about 40 to about 90% ethanol; and (b) a buffering agent to maintain a pH from about 6 to about 9.
19. (Currently Amended) A method of preparing an RNA sample substantially free of genomic DNA, comprising the following steps:
- (a) forming a lysate from a biological sample;
  - (b) contacting a pre-filtration column with said lysate, wherein said pre-filtration column comprises a fiber material, wherein said fiber material has at least one layer of glass or

Appl. No. 10/693,428  
Amendment dated February 26, 2007  
Reply to Advisory Action of January 22, 2007

borosilicate fiber; and whereby essentially all genomic DNA in said lysate is removed to produce a filtrate;

(c) forming an RNA-containing precipitate by adding an organic solvent to said filtrate from step (b);

(d) ~~contacting an RNA isolation membrane column with said RNA-containing precipitate from step (c)~~ with a polymeric membrane having a particle retention of up to about 10  $\mu\text{m}$ , wherein said ~~RNA isolation membrane column comprises a polymeric RNA isolation membrane acting~~ acts as a passive physical barrier to said RNA-containing precipitate and retaining the RNA-containing precipitate for purification; and

(e) collecting said RNA-containing precipitate from said RNA isolation membrane column, wherein said RNA-containing precipitate is substantially free of said genomic DNA.

20. (Original) The method of claim 19, wherein said fiber material has a particle retention ranging from about 0.1  $\mu\text{m}$  to about 10  $\mu\text{m}$ .

21. (Original) The method of claim 19, wherein said fiber material has a thickness ranging from about 50  $\mu\text{m}$  to about 2000  $\mu\text{m}$ .

22. (Previously presented) The method of claim 21, wherein said fiber material has a specific weight ranging from about 75  $\text{g}/\text{m}^2$  to about 300  $\text{g}/\text{m}^2$ .

23. (Currently Amended) The method of claim 19, wherein said ~~RNA isolation~~ polymeric membrane has a particle retention ranging from about 0.1 to about 10  $\mu\text{m}$ .

24. (Currently Amended) The method of claim 19, wherein said ~~RNA isolation~~ polymeric membrane is selected from the group consisting of polysulfone treated with

Appl. No. 10/693,428  
Amendment dated February 26, 2007  
Reply to Advisory Action of January 22, 2007

hydroxypropylcellulose, poly(vinylidene fluoride), nylon, nitrocellulose, polysulfone, polysulfone and poly(vinylpyrrolidone), poly(vinylpyrrolidone), and composites thereof.

25. (Withdrawn) A kit for isolating RNA in a form essentially free from genomic DNA, comprising the following: (a) at least one pre-filtration column, wherein said pre-filtration column comprises a fiber material, wherein said fiber material has at least one layer of glass or borosilicate fiber; (b) at least one RNA isolation membrane column, wherein said membrane column comprises a polymeric membrane; (c) reagents for both (a) and (b); and (d) instructions for isolating RNA with (a) through (c).

26. (Withdrawn) The kit of claim 25, wherein said RNA isolation membrane is selected from the group consisting of polysulfone treated with hydroxypropylcellulose, poly(vinylidene fluoride), nylon, nitrocellulose, polysulfone, polysulfone and poly(vinylpyrrolidone), poly(vinylpyrrolidone), and composites thereof.

27. (Withdrawn) The kit of claim 25, wherein said reagents include at least one organic solvent and a lysis buffer.